Dkt. #691-C-PCT(CN)-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	JIN, et al.
U.S. Serial No.	:	10/517,122
Filed	:	December 2, 2004
For	:	HAZARD-FREE MICROENCAPSULATION FOR STRUCTURALLY DELICATE AGENTS, AN APPLICATION OF STABLE AQUEOUS-AQUEOUS EMULSION

Law Offices of Albert Wai-Kit Chan, LLC
World Plaza, Suite 604
141-07 20th Avenue
Whitestone, New York 11357

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir/Madam:

CERTIFICATION OF TRANSLATION

My name is Tuo Jin. I graduated from University of Toronto with a Doctor of Philosophy in Pharmaceutics in 1997. I also graduated from NanKai with a Bachelor of Science degree in Chemistry in 1979, and from Hokkaido University with a Doctor of Science Degree in Physical Chemistry in 1985. I have extensive knowledge and experience in Pharmaceutics and Drug delivery and a qualified language background in English and Chinese.

I am fluent and knowledgeable in the official written languages of English, Chinese, and Japanese.

I hereby certify that the attached (**Exhibit A**) is an accurate English translation of the abstract of Chinese Patent No. 1054009A, which was previously submitted in the May 26, 2005 supplemental Information Disclosure Statement.

Respectfully submitted,

Translator's Signature

T. Jin

Translator's Printed Name

Aug. 5, 2005

Date

Address: 445 Valentine Street

Tel: 732-828-7277
Fax:

Application Number: 91101017.3
A61K 9/58

Publication date: 1991.8.28

Application date: 91.2.12
Priority: [32]90.2.13 [33] JP [31] 33/90

Applicant: Taketa Pharmaceutical Co.

Address: Osaka, Japan

Inventors: Okata ( ), Inoue ( ), kokawa ( )

Patent agency: China International Trade Promotion Committee Patent Department

Agent: Tang, WeiJie

Patent description: 14 pages

Title of Invention: Sustained Release Microcapsule

Abstract:

This invention describes a microencapsule that offers a zero order release of bioactive peptides for at lest two months. The microcapsules may be prepared though a water-in-oil emulsion with 20-70 w/w% polypeptides contained in the water phase. The oil phase contains polymers of 7000-30,000 in weight-average molecular weight and 80/10 (80/20?)-100/0 in lactide/glycolide ratio. The method includes the step of converting the water-in-oil emulsion to microcapsules.

Claims

1. A microcapsule that offers zero order release of bioactive peptides for at least two months, comprising: (a) being prepared through a water-in-oil emulsion; (b) a water phase containing 20-70 w/w% polypeptides; (c) an oil phase containing copolymer or polymer 7000-30,000 in weight-average molecular weight and 80/10 (80/20?)-100/0 in lactide/glycolide ratio; (d) converting the emulsion to microcapsules.
2. Microcapsule of claim 1, comprising the step of dispersing the emulsion into a water phase, and enabling the microcapsule to be dried in a water-in-oil-in-water three-phase system.
3. Microcapsule of claim 1, wherein the polypeptides containing two to several amino acids with molecular weight is about 200-100,000.
4. Microcapsules of claim 1, wherein the polypeptides are LH-RH or its analogues, water soluble and 1,000 or larger in molecular weight.
5. Microcapsules of claim 1, wherein the polypeptide is (pyridine) – NHC2H, (TAR-144).
6. Microcapsule of claim 1, wherein the polypeptide is TRH.
7. Microcapsule of claim 1, wherein the lactide/glycolide ratio is 90/10-100/0.
8. Microcapsule of claim 1, wherein the polymer is polylactic acid 7,000-25,000 in weight-average molecular weight.
9. Microcapsule of claim 1, wherein the inner water phase contains 25-65 w/w% polypeptides mentioned above.
10. Microcapsule of claim 1, wherein the inner water phase contains 35-60 w/w% polypeptides mentioned above.
11. Microcapsule of claim 1, wherein the inner water phase contains no drug-lagging materials.
12. A method of preparing microcapsules for zero order release for at least two months, comprising: (a) being prepared through a water-in-oil emulsion; (b) a water phase containing 20-70 w/w% polypeptides; (c) an oil phase containing copolymer or polymer 7000-30,000 in weight-average molecular weight and 80/20-100/0 in lactide/glycolide ratio; (d) dispersing and drying the water-in-oil emulsion in water [The original Chinese sentence is badly written].

13. Method of claim 12, comprising dispersing the water-in-oil emulsion in water to form a water/oil/water three-phase system for drying [extracting organic solvent] and phase separation [making the oil phase to particles].
14. Method of claim 12, wherein the water-in-oil emulsion is dispersed in an aqueous phase containing polyvinyl alcohol as surfactant.
15. Method of claim 12, wherein the polypeptide consists 2 to several amino acids, and the polymer possesses weight-average molecular weight of 200-100,000.
16. Method of claim 12, wherein the polypeptides are LH-RH or its analogues, water soluble and 1,000 or larger in molecular weight.
17. Method of claim 12, wherein the polypeptides is (pyridine) – NHC2H, (TAR-144).
18. Method of claim 12, wherein the polypeptides is TRH.
19. Method of claim 12, wherein the lactide/glycolide ratio is 90/10-100/0.
20. Method of claim 12, wherein the polymer is polylactic acid 7,000-25,000 in weight-average molecular weight.
21. Method of claim 12, wherein the inner water phase contains 25-65 w/w% polypeptides mentioned above.
22. Method of claim 12, wherein the inner water phase contains 35-60 w/w% polypeptides mentioned above.
23. Method of claim 12, wherein the inner water phase contains no drug-lagging materials

Patent Description

Sustained-release Microcapsules

This invention is regarding microcapsules for sustained release of bioactive peptides.

There are various designs for drugs that require sustained release. For example, an un-issued Japanese patent application (Toku-Kai Sho) 57-118512 and its corresponding EP-A-0052510 described a phase separation method of using miniral oil and vegetable oil to prepare microcapsules; Toku-Kai Sho 60-100516 (and corresponding US patent 4652441 and 4711782), 62-201816 (and corresponding EP-A-01901833) and 63-4146 described aqueous drying method to dry the microcapsules. According to these methods, drugs can be effectively encapsulated in microcapsules, and achieve sufficient, low burst release.

Microcapsules as a drug dosage form can meet the requirements in delivery various types of bioactive ingredients that need to be protected by microcapsules.

There numerous reports on soluble-drug containing microcapsules prepared using biodegradable polymers. However, for the category of molecularly large polypeptides, retarded release in the initial step due to low diffusion coefficient prior to polymer degradation or swelling, and severe initial burst due to preparation methods are problems for drug administration. Especially for those long period of release system, drug release in high accuracy and constant rate is an important goal. No a microcapsule technology can meet this requirement.

To meet these requirements, inventors of this patent carried out detailed study in order to find a pharmaceutical composition for long period of sustained release of bioactive peptides. The inventors realized that by selecting molecular weight of polylactide-glycolide (00/0 to 80/20 in ratio), capsules of long period and good release kinetics can be achieved. This invention provides a ground for further studies leading this goal.

In details, the primary objective of this invention is to provide a microcapsule that offers zero order release of bioactive peptides for at least two months. It is prepared through a water-in-oil emulsion, in which the inner water phase contains 20-70 w/w% polypeptides and the oil phase contains copolymer or polymer 7000-30,000 in weight-average molecular weight and 80/10 (80/20?)-100/0 in lactide/glycolide ratio. Such water-in-oil emulsion is then formed to microcapsule.

According to this invention, a microcapsule that release bioactive polypeptides in zero order form for at least two months is prepared.

The bioactive polypeptides comprise those having two to several amino acids with molecular weight approximately 200 to 100,000.

Examples of these polypeptides include LH-RH and its analogues. For LH-RH like materials [refereed to US Patent 3,853,837; 4,118,209; 3,972,589; and 4,234,571; British Patent 1,423,083; Proceedings of the National Academy of Sciences of the united States of America, Volume 78, P 6509-6512 (1981)] and LH-RH 拮抗剂 (referred to US Patent 4,086,219; 4,124,577; 4,253,997; and 4,317,815). It also includes 崔乳激素, 促肾上腺皮质激素 adrenocorticotrophic hormone (ACTH), 粗黑激素 melanocyte stimulating hormone (MSH), 促甲状腺释放激素 thyrotropin (TRH), their slats and derivatives (referred to Toku-Kai Sho 50-121273; 51-116465), 促甲状腺激素 thyrotropin-releasing hormone (TSH), 促黄体激素 luteinizing hormone (LH), 促卵泡激素 follicle stimulating (FSH), vasopressin and its ramifications,(ligamentum vasopressin et al), oxytocin, (OXT), calcitonin, CT）：(Parathormone, PTH）and its ramifications, (ref Toku-Kai Sho 62-28799), Gastrocoel, pancreozymin, vasoactive intestinal peptide (VIP), atrial natriuretic peptide (ANP), endothelin, Secretin, pancreozymin, cholecystokinin, angiotensin, angiotensin I, angiotensin II, human placenta lactogen (hPL), ）也称人类绒毛膜促生长催乳素（human chorionic somatamammotropin，hCS），人绒毛膜促性腺激素（human chorionic gonadotropin，hCG）脑啡肽, encephalin，脑啡肽衍生物 encephalin derivatives，β-内啡肽 (β-endorphin, β-EP)，京都啡肽胰岛素， 生长素释放激素（growth hormone releasing hormone,GHRHA）生长抑素（生长素释放抑制素,growth hormone release-inlease-inhibiting hormone,GHRIH,或 somatostatin）and its ramifications, (ref US patent

4,087,390,4,093,574,4,100,117 和 4253，998) 生长素 growth hormone and all kinds of cell cytokine enhancing and differentiation 胰岛素样生长因子(insulin-like growth factor,IGF) 表皮生长因子 epidermal growth factor 成纤维细胞生长因子 Fibroblast growth factor **神经生长因子 nerve growth factor** 肝细胞生长因子(hepatcoytegrowth factor, HGF 转化生长因子（transforming growth factor, TGF）骨形态发生蛋白(bone morphogenetic protein,BMP) 血管生成因子（angiogenesis factor），血小板衍生的内皮细胞生长因子（platelet－derived endothelial cell growth factor,PD－ECGF）血管生长素（angiogenin）α-干扰素(interferon α,IFN-α)、β-干扰素(interferon β,IFN-β)和 γ-干扰素(interferon γ,IFN-γ)白细胞介素(interleukin，ILI-VII)胸腺生成素（thymopoietin ）胸腺肽（ThymicPeptide） 胸腺素 Thymosins 胸腺刺激素 Thymostimulin 胸腺体液因子 thymic humoral factor.and its ramifications, (ref US patent 4,229,438)and otherThymosins (ref Proc……..) 肿瘤坏死因子 tumor necrosis factor,TNF，巨噬细胞集落刺激因子(macrophage colony stimulating factor,M-CSF) 胃动素(motilin)，促红细胞生成素 Erythropoietin，强啡肽 Dynorphin 蛙皮素 Bombesin 缓激肽(bradykinin, BK) 尿激酶 urokinase 前尿激酶（Pro-urokinase 组织型纤溶酶原活化剂（tissue-type Plasminogen Activator, tPA）and its ramifications, (ref) 链激酶 Streptokinas P 物质 **Substance P** 多粘菌素(Polymyxin) 粘菌素 Colistin 杆菌肽. Bacitracin 短杆菌肽（granmicidin）LH-RH 受体拮抗剂（LH-Lutenising hormone，黄体生成激素；RH-Releasing hormone，释放激素）。后叶加压素, 后叶加压素 derivatives (韧带加压素, for example), 崔产素, calcitonin,

According to the type, efficacy and administration period of polypeptides, the dose of these bioactive polypeptides varies widely, thus the microcapsule dose can be 0.01 mg ~ 5g, with a preference of 0.1 mg ~ 2g. Its concentration inside of the microcapsule depends on physical chemical properties of the drug with a scope of roughly 0.01% ~ 50% (w/w), and 0.1%~30% (w/w) as preferred.

The concentration of above polypeptides inside of the inner aqueous phase of the microcapsule depends on its physical chemical properties, such as solubility, with a scope of 20%~70% (w/w), preferred scope of 25%~65% (w/w), and most preferred scope of 35%~60% (w/w).

The polymers used as sustained release matrix include acid-end copolymer or polymer of lactide/glycolide. It is slightly soluble or insoluble, and biocompatible. The (lactide/glycolide) ratio varies according to required sustained release period. The selectable scope is 100/0-80/20, with preference of 100/0-90/10, and the best of 100/0.

The lactic acid can be L-, D- and DL form, with preference of the copolymer of polymer condensed from DL-lactic acid monomer or oligomer.

For DL-lactide/glycolide copolymer or polymer, the better choice is those that are synthesized by condensation without using catalyst (referred to Toku-Kai Sho 61-285215). Its polydispercity (weight-average molecular weight over number-average molecular weight) is 1.5-3.0, with preference of 1.5-2.5.

The lasting period of continuous sustained release of the microcapsules in this invention depends on molecular weight of the polymer and its lactide/glycolide ratio. For example, for zero order release of at least three months, the preferred weight-average molecular weight is 7,000-25,000 at lactide/glycolide ratio of 100/0, while the value becomes 7,000-30,000 at the lactide/glycolide ratio of 90/10, and 12,000-30,000 at 80/20.

In this document, the weight-average molecular weight and polydispercity are measured using gel permeation chromatography with a commercially available polyxyline standard.

Upon preparation of the microcapsules, the polymer concentration in the oil phase is selected between 0.5-90% (w/w), with preference of 2-60% (w/w).

The polymer solution (oil phase) is the polymer and its organic solvent.

The organic solvent above can be any organic solvent with boiling point less or equal than 120 C and immiscible with water. Some examples are halides (such as dichloromethane, chloroform, ethane chloride, ethane tri-chloride and carbon tetrachloride), ethyl acetate, ether, benzene, toluene. Its can also be binary or multi-mixture of the above solvents.

In the present invention, drug-lagging materials are in general not used in preparation of low initial burst microcapsules, but may be used depends on situation. The drug-lagging materials is for raising the viscosity of the inner water phase, or for improve solubility through ionic solubility enhancement, or cationic basic compounds that interact with the polymer to enlarge the viscosity of the oil phase.

The drug-lagging materials mentioned above include, 明胶, agarose , alginic acid, polyvinyl alcohol, or basic amino acids such as 精氨酸, lysine, peptides containing basic amino acids, organic bases such as N-methyl chitosan, and natural or synthetic polymer.

These compounds can be used alone or as mixture. Its concentration depends each compound. The preferred concentration range in the inner aqueous phase is 0.05-90%(w/w), and 0.1-80% (w/w) for even better.

The conventional methods to control release rate from microcapsules include varying hydrolysis rate [Biomaterials Vol S, 237-240 (1984)] and dispersing soluble compounds into the matrix of microcapsules to increase diffusion channels. However, the former tends to reduce release period, and the later leads to initial burst. These methods are unlikely to offer a zero order release [Chem. Pharm. Bull. Vol 36(4) 1502-150791988]]. In addition, side effects due to increased initial blood concentration are a concern for the later. Another method to control the release kinetics is to adjust

glycolide content in lactide/PLGA. This method, however, increases the hydrolysis rate and therefore reduces the period of sustained release.

The microcapsules of the present invention are prepared as following:

First, bioactive polypeptides are dissolved in water to the concentration discussed above. If necessary, drug-lagging agents, such as 明胶 or basic amino acids, can be added to form a solution or suspension that forms the inner aqueous phase.

To improve stability or solubility of the polypeptides, pH modulators may be added into the inner aqueous phase. These include carbonic acid, acetic acid, oxilic acid, citric acid, phosphoric acid, hydrochloric acid, sodium hydroxide, 精氨酸, lysine and their salts. As polypeptide stabilizers, abumin, 明胶, citric acid, EDTA, dextrin, sodium hydrosulfate, or oligo-alcohols such as PEG. Or preservatives can be added. These agents include p-hydroxyl benzoate (such as methyl p-hydroxyl benzoate, propyl p-benzoate), 卡醇, chlorine butanol, or ethyl di 甲酸 防腐剂

The inner aqueous phase obtained as above is then added into the polymer solution (the oil phase) and emulsified to the water-in-oil emulsion.

Some known effective methods are used in emulsification. These methods include stepwise stirring (screw stirring mixer, turbine stirring mixer), gel milling, homogenizing, and ultra-sonication.

The water-in-oil emulsion prepared as above is then formed microcapsules using a water-in-oil-in-water method to disperse the organic phase and extract the organic solvent from the dispersed phase [the above is the translator's understanding. The Chinese doc. is written very badly]. During the solvent extraction process, the water-in-oil emulsion is added in a third water phase to form a W/O/W three-phase emulsion. The organic solvent of the oil phase is removed by evaporation, and the microcapsules are obtained.

Adding emulsifiers into the outer water phase may stabilize this (three-phase, by translator) emulsion. The emulsifiers include anionic surfactants (such as 油酸纳，硬脂酸纳，十二烷基硫酸钠), non-ionic surfactants (such as polyethylene oxide 山梨醇，fatty acid esters, Twuen-80, Twuen-60, products of Atlas, Powder Co), derivatives of polyethylene oxide castor oil (HCO-60, HCo-50, of Nikko Chemicals Co.), PVP, PVA, carboxyl methyl cellulose, phasphotidyl lipids, or 明胶. These emulsifiers may be used individually or as mixtures. The concentration of the emulsifiers is selected from 0.01%-20%, with preference in the range of 0.5%-10%.

Any conventional method may be used to evaporate the solvent in the oil phase. These include low pressure evaporation under screw-stirring or magnetic stirring, or using a rotavapor with adjusted vacuum. When the polymer phase is solidified to certain stage, temperature of the W/O/W system may be raised to facilitate solvent evaporation.

The obtained microcapsules are collected by centrifugation or filtration, washed with distilled water to remove free bioactive peptides, drug-lagging materials, emulsifiers adsorbed on the surface of the microcapsules. At the last, the obtained microcapsules are re-dispersed in distilled water, followed by freeze-drying to remove moisture and solvent resdues.

Microcapsules may also be prepared by adding flocculants to precipitate and solidify the polymer.

Flocculants can be any polymer, mineral oil, plant oil compounds which are soluble in the solvents. These include silicone oil, sesame oil, soybean oil, corn oil, cotton seed oil, coconut oil, flax seed oil, mineral oil, n-hexane, and n-heptane. These flocculants may also be used as binary or multi-mixtures.

The microcapsules collected as above are washed repeatedly (for example using heptane) to remove undesired solvents in the polymer matrix. Further, free drugs are removed using the similar method as water-extraftion method, followed by separation of the solvent and adding aggregation preventing agents to prevent microcapsule aggregation in washing.

The microcapsules prepared using the water-extraction method in this invention showed longer and more stable sustained release profiles.

The microcapsules prepared according to this invention may be used for injectables, implants, as well as rectum or uterus absorbents.

The microcapsules prepared as above may need slightly cracking and filtration to remove oversized microcapsules. The average particle size is in the range of 0.5-1000 um with idea range of 2-500 um. When the microcapsules are used as injectable suspensions, the particle sizes have to meet the requirement, say the idea range is around 2-100 um.

The microcapsules prepared as present invention possess a number of advantages. No aggregation and attachment during preparation, and any satisfied size may easily be achieved.

The microcapsules prepared as the present invention may be administrated by injection or implanting intramuscularly, subcutaneously intravenously or to organs or diseased sites (tumor for example). They may also used for other administration routes or as material to prepare other dosage form.

In the case of formulating injectables, the microcapsules prepared in this invention are dispersed into water together with dispersing agents (Twuen-80, HCO-60, carboxyl methyl cellulose, sodium alginate), preservatives (p-hydroxyl methyl benzoate, p-hydroxyl propyl benzoate), tonicity keepers (NaCl, minotol, 山梨醇，glucose). Or they can be mixed with plant oils such as sesame oil or corn oil and suspended to water to formulate to suspension dosage form for sustained release.

The microcapsules can also be mixed with excipients (such as minitol, 山梨醇, lactose, glucose), distilled water and other dispersers to remixed, followed by freeze-drying or spray-drying for solidification. This operation may turn the microcapsule injectables to more stable sustained release dosage form.

The dosage size of this sustained release dosage form depends upon the type of the bioactive polypeptides, their [original doses], formulation, required period of sustained release, animal (such as mice, rats, rabbits, sheep, pigs, crow, horses, and human), and purpose of drug therapy. Doses should be kept within the scope of effective level. For example, a single dose for an animal is selected from 0.1 – 100 mg/Kg, with preference of 0.2-50 mg/Kg.

Microcapsuels prepared in the present method show more efficient drug efficacy of bioactive polypeptides than single dose formulation. The polymer is biocompatible and the microcapsules may continuously release drug from long period.

The mirocapsules of this invention possess following characteristics: (1) all dosage forms may provide continuous, sustained release of bioactive polypeptides for three months, or an injection for six months, and achieve idea and stable drug therapy without daily injection. The release period is longer than conventional sustained release dosage forms. (2) Injectables formulated with biodegradable polymers no longer need surgical operation as for implant. Like conventional suspension dosage forms, this formulation can easily administrated through subcutaneous, intramuscular, or organ-targeted, or disease sites injection. And there is no need to remove the formulation after depletion of the loaded drugs.

Following examples provide description of this invention in more details.

Reference Example 1

An 85% aqueous solution of DL-lactic acid, 160g, was added into a 4-neck bottle equipped with thermometer, condenser, and nitrogen introducer. The solution was then heated in nitrogen atmosphere under reduced pressure for six hours to remove water. The scope of the temperature and pressure inside of the bottle was 105 C and 350 mmHg to 150 C and 30 mmHg. Reaction was carried out at 175 C under reduced pressure (3-5 mmHg) for 90 hours, followed by cooling down to room temperature. About 980g colorless polymer was obtained. This polymer was dissolved in THF, and measured by gel permeation chromatography, with commercially available polyxylene standard, for weight-average molecular weight and polydispersity. The values were 17,200 and 1.89, for molecular weight and polydispersity, respectively.

Example 1

TAR-144, 400 mg, was dissolved in 0.5 ml distilled water.

This solution was added into 7.5 ml dichloromethane solution of DL-polylactic acid, containing 4g of the polymer. [weight-average molecular weight was 18,000 for Lot No. 870818 (microcapsule Lot no. 244,245), and 18,200 (with polydispersity of 1.76) for Lot No. 880622 (microcapsule Lot No. 248)]. This sample was homogenized using a small homegenizer (Polyron, from Kinematica, Switzerland) for 60 seconds to form a water-in-oil emulsion. After cooling down to 15 C, this emulsion was added into 1000 ml pre-cooled aqueous solution containing 0.25% PVA. The sample was stirred with the small homogenizer to form a water/oil/water emulsion. Then, dichloromethane was evaporated under stirring, leading solidification of the dispersed water-in-oil phase. The solidified materials were collected by centrifugation.

The collected materials were re-suspended in distilled water to wash away the [un-encapsulated] drug and emulsifier.

The re-collected microcapsules were subjected to freeze-drying to remove solvent residues and moisture, leading to dehydrated powder-like product. The microcapsules contained 9% drug with encapsulation efficiency of 100% or more.

The prepared microcapsules were injected to rats (n=5) subcutaneously. The release rate was measured by determining TAP-144 content within the microcapsules at the injection site. Result is shown in Table 1.

Table 1, In vivo release rate.

	Drug retained at subcutaneous injection site (%)				
Lot	day 1	week 2	week 4	week 8	week 14
244	102.2	89.0	70.2	44.0	9.5
245	105.9	82.4	69.4	52.1	9.8
248	104.1	75.4	72.8	43.7	11.6

These microcapsules did not show a initial burst. The detectable TAP-144 release lasted for 14 week, i.e. 3 months, with good reproducibility.

Example 2

Similarly, 400 mg of TAR-144 was dissolved in 0.5 ml of distilled water, and at the same time, 4 g of poly-DL-lactic acid (weight-average molecular weight: 8,400, Lot. 870304, microcapsule Lot. 312) was dissolved in 5 ml dichloromethane. The two solutions were mixed as above mentioned method to form a water-in-oil emulsion. This emulsion was cooled to 13 C and added into 1000ml of 0.25% PVA aqueous solution as above [Example 1, translator] to form a water/oil/water emulsion. Microcapsules were therefore obtained.

Three of each 550 mg of TAP-144 was dissolved respectively in 1 ml distilled water. For the polymer phase, poly-DL-lactic acid of three different Lots, each 4 g, were dissolved in each 7.5 ml dichloromethane, respectively. The three aqueous solution were added respectively into each dichloromethane solution of polymers as above mentioned procedure to form three water-in-oil emulsions. Each of the three obtained water-in-oil emulsions were dispersed, respectively, in 1000 ml (100 ml in the original, and it may be a typo) 0.25% PVA solution. For the first sample (Lot. 402?), the temperature of the PVA solution was 15 C, and was 18 C (the original said 180C. That is absolutely a typo because water cannot be heated to 180C) for the other two. The rest of the steps were the same as previous examples. Obtained microcapsules had loading efficiency of 101%, 113% and 103%, respectively.

Table 2 shows the in vivo drug release rate from microcapsules prepared as above.

Table 2:

Drug retained at subcutaneous injection site (%)

Lot.	n	day1	week1	week2	week8	week12	week14
312	5	86.3	82.2	41.2	9.8	---	---
402	3	98.0	78.2	64.9	38.4	20.0	---
405	5	88.8	79.4	52.2	33.8	---	21.3
406	5	85.5	86.2	56.7	38.8	---	23.1

With slight initial burst, the drug was released continuously for two months. The release period depends on hydrolysis rate of the polymer used.

Example 3:

Aqueous solution of Tap-144 and dichloromethane solution of PLGA were prepared using the procedure same to that in Example 1. Two TAP-144 solutions were prepared: 400 mg TAP-144 was dissolved in 0.5 ml distilled water, and 550 mg of the same drug was dissolved in 1 ml distilled water. Accordingly, two PLGA solutions were prepared by dissolving 4 g of the polymer into dichloromethane. The two solutions contains PLGA (90/10) from two different Lot, [Lot No. 870320 (weight-average molecular weight = 19,000), and microcapsule Lot No. 315; and Lot No. 891020 (weight-average molecular weight = 13,800), and microcapsule Lot no. 410]. The two aqueous solutions were dispersed into the two PLGA solutions at 15 C and 18 C, respectively. For the final microcapsule products of the two samples, encapsulation efficiencies were 106% and 100%, respectively.

With the same procedure as previous examples, the microcapsule formulations were administrated to rats subcutaneously to measure in vivo drug release rate. Table 3 shows the result, indicating that the drug release continued for more than two months.

Table 3: Drug release rate in vivo (n = 5)

	Drug retained at subcutaneous injection site (%)						
Lot	day 1	week 1	week 2	week 4	week 6	week 8	week 10
315	77.4	76.0	59.2	51.6	41.1	25.8	---
410	93.5	88.3	64.1	52.5	33.1	32.7	15.4

Example 4

Based on the procedures in Example 1, 280 mg TRH (free molecular form) was dissolved in 0.25 ml distilled water. Poly-DL-lactic acid, same as that used in Example 2 (average molecular weight: 17,200, polydispersity: 1.89), was dissolved in 6 ml dichloromethane to form the oil phase [did not mention how many gram of PLA]. For the

next step, temperature of the outer water phase was 15 C. Encapsulation efficiency of the drug into the obtained microcasules (Lot No. R-103) was 85.8%.

Table 4 shows the drug release result of this microcasules that drug release lasted for 3 months.

Table 4:

	Drug retained at subcutaneous injection site (%)				
Lot	day 1	week 2	week 4	week 8	week 12
R103	98.3	80.0	61.8	30.0	6.7